[Frontiers in Bioscience E2, 1164-1168, June 1, 2010]

Telomere length and its associations with oxidized-LDL, carotid artery distensibility and smoking

Tim S. Nawrot[1,2], Jan A. Staessen[1,3], Paul Holvoet[4], Harry A. Struijker-Boudier[5], Paul Schiffers[5], Luc M. Van Bortel[6], Robert H. Fagard[1], Jeffrey P. Gardner[7], Masayuki Kimura[7], Abraham Aviv[7]

[1]Study Coordinating Centre, Laboratory of Hypertension, Department of Molecular and Cardiovascular Research, University of Leuven, Leuven, Belgium,[2]Centre for Environmental Sciences, Hasselt University, Diepenbeek, Belgium, [3]Department of Epidemiology, Maastricht University, Maastricht, The Netherlands. [4]Cardiovascular Research Unit, Center for Experimental Surgery and Anesthesiology, University of Leuven, Leuven, Belgium,[5]Department of Pharmacology and Toxicology, Cardiovascular Research Institute Maastricht, Maastricht University, the Netherlands, [6]Heymans Institute of Pharmacology, Ghent University, Gent, Belgium, [7]The Center of Human Development and Aging, University of Medicine and Dentistry of New Jersey, New Jersey Medical School, Newark, NJ, USA.

TABLE OF CONTENTS

1. ABSTRACT

Oxidative stress is a key factor driving the aging of cells and arteries. Studies suggest that white blood cell (WBC) telomere length is an index of systemic aging. We, therefore, investigated the association between WBC telomere length and oxidized-LDL, and vascular aging, expressed by the distensibility of the carotid artery. We studied a random population sample of 216 non-smokers and 89, smokers. In all subjects, age and gender- adjusted telomere length was inversely correlated with plasma oxidized-LDL (regression coefficient = -0.656 kb/mg/dL; p=0.0006). Independent of gender, age and mean blood pressure, carotid distensibility increased with telomere length (2.33±1.18 10^{-3}/kPa/kb; p=0.05) but decreased with higher plasma levels of oxidized LDL (-10.7±3.91 10^{-3}/kPa/ mg/dL; p=0.006). Adjusted for gender and age, smokers' telomere length was shorter (6.72 *vs* 6.91 kb; p=0.014) and plasma oxidized-LDL level higher (0.52 *vs* 0.46 mg/dL; p=0.03) than in non-smokers. Higher level of oxidized-LDL, is associated with shorter WBC telomeres and increased stiffness of the carotid artery. Smoking is marked by increased oxidative stress in concert with shortened WBC telomere length.

2. INTRODUCTION

The aging of the vasculature is a complex process that in large measure reflects the overall aging of the individual. Oxidative stress is somehow involved in this process. (1). We explored the proposition that systemic oxidative stress, expressed in oxidized-LDL, might be a factor that is common to both systemic and vascular aging. To this end, we used white blood cell (WBC) telomere length as an index of systemic aging (2) and the common carotid artery distensibility as a measure of vascular aging. As aging reflects the input of both genetic and environmental factors, we examined the effect of cigarette smoking— an important environmental source of free radicals (3) that might accelerate aging (4) — on WBC telomere length, oxidized-LDL and carotid distensibility.

3. MATERIALS AND METHODS

3.1 The cohort

We studied 305 subjects (55.8% women), who were randomly recruited from the population of a geographically defined area within the framework of the Flemish Study on Environment, Genes and Health

Outcomes (FLEMENGHO). Each volunteer donated a fasting blood sample and underwent a physical examination and carotid artery distensibility measurements. Using a standardized questionnaire, we collected information about personal and familial medical history, smoking and drinking habits, and the use of medications. The study was performed in accordance with the Helsinki Declaration. The Ethics Committee of the University of Leuven approved the protocol.

3.2. White blood cell telomere length measurements

WBC telomere length was measured from the mean of the terminal restriction fragment length, as described before (5). Briefly, DNA samples were digested with *Hinf*I and *Rsa*I (Roche Diagnostics Corporation, Indianapolis, US) and resolved on 0.5% agarose gels. DNA was transferred to a positively charged nylon membrane and telomeric DNA detected by Southern hybridisation to a digoxigenin 3'-end labeled 5'-$(CCCTAA)_3$ after overnight incubation at 65° C. The labelled DNA was visualised using a digoxigenin luminescent detection procedure and exposure on X-ray hyperfilm (Amersham Biosciences, UK). Telomere length of each sample was the average of duplicate measurements. If the duplicate for a sample varied by 5% or more, we repeated the measurement and took the mean of the two that were less than 5% apart. This occurred in less than 5% of the samples. To control for possible variation between batches, we ran internal standards on each gel. The coefficient of variation averaged 1.7%. The laboratory conducting the telomere length measurements was blinded to all characteristics of the WBC donors. Results of telomere measurements, identified only by coded ID numbers, were electronically transmitted and merged with the covariate data at the Study Coordinating Centre, Laboratory of Hypertension, Department of Molecular and Cardiovascular Research, University of Leuven.

3.3. Oxidized-LDL measurements

Venous blood (5mL) was collected into 100 µL buffer containing 0.1 mgm/L citrate, 1 mmol/L EDTA, 20 µmol/L vitamin E, 10 µmol/L butylated hydroxytoluene, 20 µmol/L dipyridamole, and 15 mmol/L theophylline. The blood sample was spun at 3000*g* for 15 minutes at room temperature. Within 25 minutes, the supernatant was stored at -80°C until assayed. We used a monoclonal antibody 4E6–based competition ELISA for measuring the plasma levels of oxidized LDL(6,7). The coefficient of variation of oxidized LDL was 12%. LDL cholesterol, HDL cholesterol and triglycerides were measured by automated enzymatic methods (Boehringer, Mannheim, Germany). We calculated LDL cholesterol from LDL cholesterol and triglycerides by means of Friedewald's formula.

3.4. Distensibility of the common carotid artery

Vascular measurements were performed after the individual had rested in the supine position for 15 minutes. An experienced researcher (TN) performed the measurements using a pulsed ultrasound wall tracking system (Wall Track System, Pie Medical, Maastricht, The Netherlands), which has been validatedl (8). Measurements were taken at the common carotid artery 2 cm proximal of the carotid bulb. We used applanation tonometry with a pencil-shaped probe (Millar Instruments, Houston, TX) to calibrate the carotid pulse wave to the diastolic and mean arterial pressure at the level of the brachial artery, which were measured with a semiautomated device (Omron HEM 705CP, Kyoto, Japan). If tonometry was impossible due to obesity or the presence of arterial plaque in the common carotid artery, the vessel wall movement contour was used as a surrogate for the tonometrically derived pulse pressure contour with calibration as described above (9). The distensibility coefficient (DC) was derived from the diastolic cross-sectional area (A), the systolic increase in cross-sectional area (ΔA) and the local pulse pressure (ΔP) according to the formula: $DC= (\Delta A/A)/\Delta P$. A and ΔA were calculated as $A= \pi \times (D/2)^2$ and $\Delta A= \pi \times [(D+\Delta D)/2]^2 - \pi \times (D/2)^2$.

3.5. Statistical Analysis

We used SAS software version 8.1 (SAS Institute Inc, Cary, NC) for database management and statistical analysis. For comparison of means and proportions, we applied Student's t-test and the chi-statistic, respectively. We searched for possible covariates of the phenotypes under study by stepwise regression with the p values for independent variables to enter and stay in the model set at 0.05. In multiple means tests, we applied Bonferroni's correction to adjust the significance levels.

4. RESULTS

4.1. Characteristics of the cohort

Mean age of the 142 men and 163 women was similar and averaged 42.5 (SD: 16.5, range 12-81) years. Median daily smoking amounted to 15 cigarettes (inter-quartile range, 10-25) in 45 male smokers and 44 female smokers. Smokers and non-smokers had similar sex ratio, age, and carotid distensibility (table 1). Compared with non-smokers, smokers reported more frequently alcohol intake and displayed higher levels of serum LDL cholesterol and plasma oxidized-LDL (table 1).

4.2. WBC telomere profile

WBC telomere length decreased with age (-0.024 ±0.003 kb per year; $p<0{\cdot}0001$). Age-adjusted telomere length was shorter in men than women (6.77±0.05 kb *vs* 6.92±0.05 kb; p=0.028). In stepwise regression analysis, telomere length was independently and negatively correlated with age and was shorter in males than females and in smokers than non-smokers. These three covariates respectively explained 26.5% ($p<0.0001$), 1.2% (p=0.036) and 1.7% (p=0.011) of the variance in telomere length. After gender and age adjustments, telomere length was significantly shorter in smokers than non-smokers (6.72±0.06 *vs* 6.91±0.04 kb; p=0.014) and decreased with the number of pack years (figure 1).

4.3. Oxidized-LDL

Plasma oxidized LDL increased with age (regression coefficient ±SE, 0.0016±0.0006 mg/dL/year; p=0.008). Adjusted for age, men and women had the same level of oxidized-LDL (0.48±0.02 *vs.* 0.47±0.02 mg/dL; p=0.11), while smokers had higher levels of oxidized-LDL

Table 1. Characteristics of smokers and non-smokers

	Non-smokers (n=216)	Smokers (n=89)	p
Clinical features			
Women, No. (%)	119·0 (55·1)	45.0 (49.5)	0.37
On oral contraceptives, No. (%)	20·0 (16·8)	11.0 (24.4)	0.49
Age, years	42.1 (17.9)	46.1 (12.7)	0.51
Body mass index, kg/m^2	25.0 (4.6)	24.4 (3.2)	0.21
Systolic blood pressure, mmHg	122.9 (16.1)	125.0 (13.1)	0.27
Diastolic blood pressure, mmHg	76.6 (11·7)	77.7 (10.4)	0.44
Alcohol use, No. (%)	63.0 (29·2)	44.0 (48.4)	0.0013
Serum total cholesterol, mg/dL	208.0 (45.6)	219.0 (45.6)	0.06
Serum LDL cholesterol, mg/dL	112.0 (39.3)	118.0 (40.2)	0.07
Serum HDL cholesterol, mg/dL	52.2 (54·6)	52.5 (56.0)	0.87
Serum triglycerides, mg/dL	223.6 (125.0)	225.9 (129.3)	0.88
Phenotypes[1]			
Telomere length, kb	6.91 (0.67)	6.75 (0.67)	0.04
Plasma oxidized-LDL, mg/dL	0.46 (0.17)	0.52 (0.19)	0.02
Carotid distensibility, 10^{-3}/kPa	27.6 (15.8)	27.1 (11.9)	0.73

p comparison between smokers and non-smokers, Values are arithmetic (SD) or geometric (95% CI) means or numbering subjects (%),[1]Values are unadjusted for age P=0.03

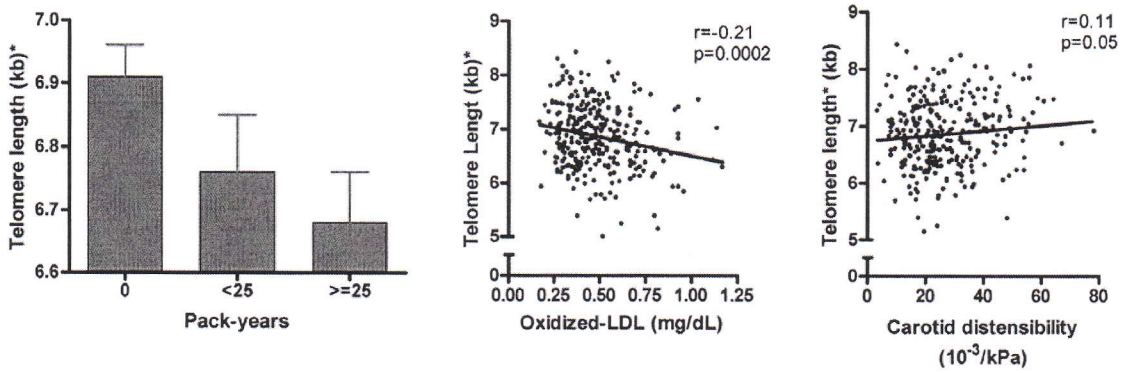

Figure 1. The relations between age-adjusted telomere length, cigarette smoking (pack-years), with oxidized –LD and carotid distensibility. *Telomere length was age-adjusted.

than non-smokers (0.52±0.01 *vs.* 0.46±0.02 mg/dL; p=0.03) and plasma oxidized-LDL increased with the number of pack years (0.0030±0.0009 mg/dL/year; p=0.0002).

4.4. Carotid artery distensibility

In stepwise multiple regression, carotid distensibility was lower in men (-3.71±1.41 10^{-3}/kPa; p=0.009) and decreased with age (-0.412±0.11 10^{-3}/kPa/years; p=0.0002) and mean arterial pressure (-0.463±0·18 10^{-3}/kPa/mmHg; p=0.012). These three covariates explained 1.5% (p=0.0094), 32.6% (p<0.0001) and 2.8% (p=0.0005) of the variance in carotid distensibility.

4.5. Associations of WBC, telomere length, oxidized-LDL and carotid distensibility

After adjustment for gender and age, a 0.11±0.03 kb shorter telomere length was associated with a 1-SD increase (0.17 mg/dL) in plasma oxidized-LDL (p=0.0006) (Figure 1). Additional adjustment for smoking did not alter this relation. Adjusted for gender, age and mean blood pressure, carotid distensibility increased with telomere length (2.33±1.18 10^{-3}/kPa/kb; p=0.05), but decreased with higher plasma levels of oxidized-LDL (-10.7±3.91 10^{-3}/kPa/mg/dL; p=0.006) (figure 2). Neither age-adjusted telomere length nor carotid distensibility were associated with lipid parameters other than oxidized- LDL.

5. DISCUSSION

Telomere attrition results from somatic cell replication and oxidative stress may further accelerates this process (10). Thus, telomere length represents a record of the replicative history of cells and *in vivo* it might also reflect the cumulative oxidative stress burden over the lifetime of the individual. A body of research supports the idea that WBC telomere length is an index of biological age (aging) in that individuals with a host of aging-related disease, marked by increased oxidative stress and inflammation, are more likely to have shortened WBC telomere length (11-17). This is particularly the case for age-related vascular disease (18,19).

Our key finding is that telomere length inversely correlates with the plasma level of oxidized-LDL, a biomolecular marker of systemic oxidative stress. In addition, age-adjusted, telomere length was 190 bp shorter in smokers than in non-smokers in agreement with previous

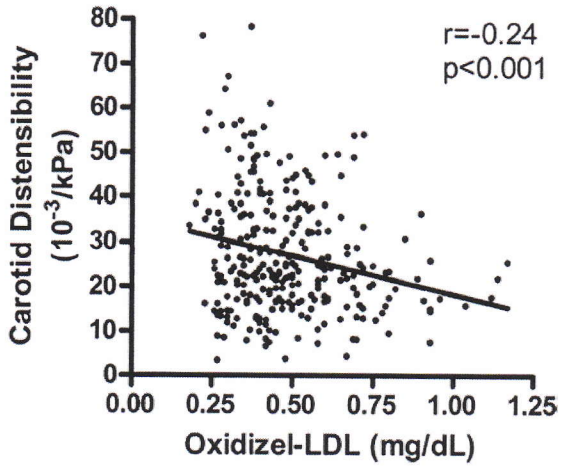

Figure 2. Association between carotid distensibility and oxidized-LDL

finding in another cohort (20). In telomeric year equivalence (based on telomere attrition rate of 0.024 kb/year), smokers were biologically older than non-smokers by roughly 8 years. This effect of smoking on telomere length corresponds with Doll's observations on mortality and smoking showing that smokers on average die about 10 years earlier than lifelong non-smokers (210) and it further supports the concept that smoking accelerates systemic aging (4).

Epidemiological studies demonstrated that tobacco smoke is a major cause of both cancer and vascular diseases (21). More than 3800 chemicals are present in tobacco smoke, which may cause oxidative stress via biotransformation, or by macrophage activation (22,23). Tobacco smoke also increases LDL oxidation and may enhance the production of small-dense LDL, which is more readily oxidized (24,25).

Arterial stiffness is an index of vascular aging and an important risk factor that independently predicts cardiovascular death and death related to other factors (26). A number of studies have examined the relations between WBC telomere length and indicators of vascular aging and cardiovascular risks in humans (14,27,28). Given that cardiovascular risks increase with age, biologically, the age of individuals with relatively short telomeres may be more advanced than their chronological age would indicate. Support for such a concept has emerged from the finding that telomere length, as expressed in WBC, is shorter in subjects with atherosclerosis than in their age-matched peers (14,29). The unifying thread for short WBC telomere length and vascular lesions may well be oxidative stress.

Statins promote potent systemic antioxidant effects in vivo through suppression of different oxidation pathways, including the generation of myeloperoxidase-derived and nitric oxide–derived oxidants (30). Recently, a beneficial effect of statins on telomere biology has been described. In all subjects, the sex and age adjusted telomere length was independently and inversely correlated with plasma oxidized-LDL. Oxidative stress is central to the aging process (30,31) and it may accelerate the rate of telomeric erosion per replicative cycle (10). While, epidemiologic studies cannot elucidate such mechanistic connections, as observed in our population-based cohort, WBC telomere length was inversely associated with plasma levels of oxidized-LDL— a finding in agreement with previous observations, which estimated oxidative stress by urinary isoprostanes (13,17). In our study, oxidative stress was reflected by oxidized-LDL. Increased LDL oxidation is associated with coronary artery disease. Circulating oxidized LDL does not originate from extensive metal ion-induced oxidation in the blood but from mild oxidation in the arterial wall by cell-associated lipoxygenase and/or myeloperoxidase (32).

We showed for the first time that in the population at large telomere length is inversely associated with plasma levels of oxidized-LDL. Thus oxidative stress and inflammation, as exemplified by oxidized LDL, likely play an important role in biological ageing, a process which may be accelerated in smokers.

6. ACKNOWLEDGMENT

The Flemish Study on Environment, Genes, and Health Outcomes was supported by research grants from the Fonds voor Wetenschappelijk Onderzoek Vlaanderen, Brussels, Belgium (G.0424.03), the University of Leuven (OT/99/28), and the European Union (IC15-CT98-0329-EPOGH and QLG-CT-2000-01137-EURNETGEN). Measurements on oxidized-LDL were funded by The Fonds voor Wetenschappelijk Onderzoek-Vlaanderen (Program G0232.05; PH) and the Interuniversity Attraction Poles Program P5/02 (PH). Telomere research at The Center of Human Development and Aging, the University of Medicine and Dentistry of New Jersey, New Jersey Medical School was funded by NIH grant AG021593 and AG020132 and a grant from the Healthcare Foundation of New Jersey.

7. REFERENCES

1. R. Stocker, J.F. Keaney: New insights on oxidative stress in the artery wall. *J Thromb Haemost* 3, 1825-1834 (2005)

2. T.S. Nawrot, J.A. Staessen, J.P. Gardner, A. Aviv. Telomere length and its possible linkt to X chromosome. *Lancet* 363, 507-10 (2004)

3. W.A. Pryor, K. Stone: Oxidants in cigarette smoke. Radicals hydrogen peroxide, pyroxynitrite, and peroxynitrite. *Ann NY Acad Sci* 686, 12-27 (1993)

4. D. Bernhard, C. Moser, A. Backovic, G. Wick: Cigarette smoke--an aging accelerator? *Exp Gerontol* 42, 160-5 (2006)

5. K. Okuda, A. Bardeguez, J.P. Gardner, P. Rodriguez, V. Ganesh, M. Kimura, J. Skurnick, G. Awad, A. Aviv: Telomere length in the newborn. *Ped Res* 52, 377-381 (2002)

6. P. Holvoet, D. Collen, W.F. Van de: Malondialdehyde-modified LDL as a marker of acute coronary syndromes. *JAMA* 281, 1718-1721 (1999)

7. P. Holvoet, T.B. Harris, R.P. Tracy, P. Verhamme, A.B. Newman, S.M. Rubin, E.M. Simonsick, L.H. Colbert, S.B. Kritchevsky: Association of high coronary heart disease risk status with circulating oxidized LDL in the well-functioning elderly: findings from the Health, Aging, and Body Composition study. *Arterioscler Thromb Vasc Biol* 23, 1444-1448 (2003)

8. A.P. Hoeks, P.J. Brands, F.A. Smeets, R.S. Reneman: Assessment of the distensibility of superficial arteries. *Ultrasound Med Biol* 16, 121-128 (1990)

9.L.M. Van Bortel, E.J. Balkestein, J.J. van der Heijden-Spek, F.H. Vanmolkot, J.A. Staessen, J.A. Kragten, J.W. Vredeveld, M.E. Safar, H.A. Stgruijker Boudier, A.P. Hoeks: Non-invasive assessment of local arterial pulse pressure: comparison of applanation tonometry and echo-tracking. *J Hypertens* 19, 1037-1044 (2001)

10. T. von Zglinicki: Role of oxidative stress in telomere length regulation and replicative senescence. *Ann N Y Acad Sci* 908, 99-110 (2001)

11. R.M. Cawthon, K.R. Smith, E. O'Brien, A. Sivatchenko, R.A. Kerber: Association between telomere length in blood and mortality in people aged 60 years or older. *Lancet* 361, 393-395 (2003)

12. A. Fitzpatrick, R.A. Kronmal, J.P. Gardner, B.M. Psaty, N.S. Jenny, R.P. Tracy, J. Walston, M. Kimura, A. Aviv: Leukocyte Telomere Length and Cardiovascular Disease in the Cardiovascular Health Study. *Am J* Epidemiol 165, 14-21 (2007)

13. S. Demissie, D. Levy, E.J. Benjamin, A. Cupples, J.P. Gardner, A. Herbert, M. Kimura, M.G. Larson, J.B. Meigs, J.F. Keaney, A. Aviv: Insulin resistance, oxidative stress, hypertension, and leukocyte telomere length in men from the Framingham Heart Study. *Aging Cell* 5, 325-330 (2006)

14. S. Brouilette, R.K. Singh, J.R. Thompson, A.H. Goodall, N.J. Samani: White cell telomere length and risk of premature myocardial infarction. *Arterioscler Thromb Vasc Biol* 23, 842-846 (2003)

15. T. von Zglinicki, V. Serra, M. Lorenz, G. Saretzki, R. Lenzen-Grossimlighaus, R. Gessner, A. Risch, E. Steinhagen-Thiessen: Short telomeres in patients with vascular dementia: an indicator of low antioxidative capacity and a possible risk factor? *Lab Invest* 80, 1739-1747 (2000)

16. L.A. Panossian, V.R. Porter, H.F. Valenzuela, X. Zhu, E. Reback, D. Masterman, J.L. Cummings, R.B. Effros: Telomere shortening in T cells correlate with Alzheimer's disease status. *Neurobiol Aging* 24, 77-84 (2003)

17. E.S. Epel, E.H. Blackburn, J. Lin, F.S. Dhabhar, N.E. Adler, J.D. Morrow, R.M. Cawthon: Accelerated telomere shortening in response to life stress. *Proc Natl Acad Sci USA* 101, 17312-17317 (2004)

18. I.K. Moon, M.B. Jarstfer: The human telomere and its relationship to human disease, therapy, and tissue engineering: *Front Biosci* 12, 4595-620 (2007)

19. M.D. Edo, V. Andres: Aging, telomeres, and atherosclerosis: *Cardiovasc Res* 66, 213-221 (2005)

20. A.M. Valdes, T. Andrew, J.P. Gardner, M. Kimura, E. Oelsner, L.F. Cherkas, A. Aviv, T.D. Spector: Obesity, cigarette smoking, and telomere length in women. *Lancet* 366, 662-4 (2005)

21. R. Doll, R. Peto, J. Boreham, I. Sutherland: Mortality in relation to smoking: 50 years' observations on male British doctors. *BMJ* 328, 1519 (2004)

22. J.F. Lesgards, P. Durand, M. Lassarre, P. Stocker, G. Lesgards, A. Lanteaume, M. Prost, M.P. Lehucher-Michel: Assessment of lifestyle effects on the overall antioxidant capacity of healthy subjects. *Environ Health Perspect* 110, 479-86 (2002)

23. E. Alfaro-Moreno, T.S. Nawrot, A. Nemmar, B. Nemery Particulate matter in the environment: pulmonary and cardiovascular effects. *Curr Opin Pulm Med.* 13, 98-106 (2007)

24. D.F. Church, W.A. Pryor: Free-radical chemistry of cigarette smoke and its toxicological implications. *Environ Health Perspect* 64, 111-126 (1985)

25. D. Harats, M. Ben Naim, Y. Dabach, G. Hollander, O. Stein, Y. Stein: Cigarette smoking renders LDL susceptible to peroxidative modification and enhanced metabolism by macrophages. *Atherosclerosis* 79, 245-522 (1989)

26. G.M. London, J.N. Cohn: Prognostic application of arterial stiffness: task forces. *Am J Hypertens* 15, 754-758 (2002)

27. A. Benetos, K. Okuda, M. Lajemi, M. Kimura, F. Thomas, J. Skurnick, C. Labat, K. Bean, A. Aviv: Telomere length as an indicator of biological aging: the gender effect and relation with pulse pressure and pulse wave velocity. *Hypertension* 37, 381-385 (2001)

28. E. Jeanclos, N.J. Schork, K.O. Kyvik, M. Kimura, J.H. Skurnick, A. Aviv: Telomere length inversely correlates with pulse pressure and is highly familial. *Hypertension* 36, 195-200 (2000)

29. A. Benetos, J.P. Gardner, M. Zureik, C. Labat, L. Xiaobin, C. Adamopoulos, M. Temmar, K.E. Bean, F Thomas, A. Aviv: Short telomeres are associated with increased carotid atherosclerosis in hypertensive subjects. *Hypertension* 43, 182-185 (2004)

30. I. Spyridopoulos, J. Haendeler, C. Urbich, T.H.

Brummendorf, H. Oh, M.D. Schneider, A.M. Zeiher, S. Dimmeler: Statins enhance migratory capacity by upregulation of the telomere repeat-binding factor TRF2 in endothelial progenitor cells. *Circulation* 110, 3136-42 (2004)

31. H. Huang, K.G. Manton: The role of oxidative damage in mitochondria during aging: a review. *Front Biosci* 1, 1100-17 (2004)

32. A. Mertens, P. Holvoet: Oxidized LDL and HDL: antagonists in atherothrombosis. *FASEB J* 15, 2073-84 (2001)

Key Words: Telomere, Smoking, Vascular Stiffness

Send correspondence to: Jan A. Staessen, Studies Coordinating Centre, Laboratory of Hypertension, University of Leuven. Campus Gasthuisberg, Herestraat 49, bus 702, B-3000 Leuven, Belgium. Tel: 32-16-34-7104, Fax: 32-16-34-7106, E-mail: jan.staessen@med.kuleuven.be

http://www.bioscience.org/current/vol2E.htm